REMARKS
EXECUTIVE SUMMARY

1. **The Korlach et al. 1999 provisional *did not* disclose beta or gamma labeled nucleotides. Therefore, Applicants need to only present documentary support to antedated the Korlach et al. non-provisional application filed on 17 May 2000.**

2. **Applicants invented their technology before 17 May 2000 and submit a Declaration and a redacted proposal submitted to a Federal Funding Agency prior to 17 May 2000.**

3. **Applicants are supplying a chart showing specific support for each claim limitation in each current claim.**

4. **Like Korlach et al., Schneider et al. did not disclose using beta or gamma labeled nucleotides and labeled polymerase in a tag interactive format such as FRET, even though their entire application is directed to interactions between a base, sugar or backbone (alpha phosphate) labeled dNTP and a labeled polymerase – a strong teach away from the present invention.**

General Comments

The 1999 Korlach et al. filing *does not* disclose beta or gamma labeled nucleotides.

Moreover, the 1999 Korlach et al. Provisional including disclosure to FRET only in the context of labeled dNTPs, where the label remains with the nucleotide upon incorporation:

> 3. Fluorescence Bursts by Fluorescence Resonance Energy Transfer and Photobleaching
>
> **Detection of fluorescence resonance energy transfer (FRET) from a donor fluorophore (e.g., a donor attached to the polymerase) to adjacent nucleotide analog acceptors that are incorporated into the growing DNA strand suggests an elegant possibility of lowering background from incorporated nucleotides since FRET only reaches very short distances including about 20 nucleotides and decays at the reciprocal sixth power of distance.** Already incorporated nucleotides further away from the donor would not contribute to the fluorescent signal since distance and orientation constraints of energy transfer reduce the effective range of observation to less than 60 Å, thereby effectively eliminating background fluorescence from unincorporated nucleotides. **However, residual resonance energy transfer between neighboring fluorophores in the DNA molecule[39] might represent a problem, which could complicate this method and exclude its practicability. Without photobleaching the method requires high sensitivity since repeat nucleotides leave the range of FRET at the same rate that new nucleotides are adding creating severe sequence recognition ambiguity.**
>
> The ideal fluorescent signal for single molecule sequencing consists of

time resolved bursts of spectrally distinguishable fluorescence as each nucleotide is bound. The expected signals of about 10^4 photons from each fluorophore can be excited at a rate that is terminated by irreversible photobleaching in about 0.1 ms. This occurs well within the average time intervals between nucleotide addition at more than one millisecond. Thus the ideal situation, a time resolved train of color resolved fluorescent bursts, could be obtained if nucleotides were bound at constant intervals. However, nucleotide binding comprises a succession of stochastic Poisson processes so that bursts would be initiated in template sequence but at random intervals so that some bursts would overlap. **It is overcoming this random overlap that introduces the greatest demands on our technology and motivates many of the refinements introduced in later presentation of the physical technologies.**

Korlach et al 1999 Provisional at Page 9, paragraphs 1&2.

1. Fluorescence Resonance Energy Transfer

The most elegant strategy to limit the number of nucleotides that simultaneously emit fluorescence is to bind donor fluorophores to the polymerase and illuminate at a wavelength that excites only the donor. The excited donor molecule transfers its energy only to nearby acceptor fluorophores, which emit the spectrally resolved acceptor fluorescence of each labeled nucleotide as it is added. **So far this FRET strategy seems basically straightforward; the first problem arises when we note that the effective resonant energy transfer distances are about 50 Å which would encompass 10-20 nucleotides. However, for each nucleotides added, the earlier addition synchronously steps down the $1/r^6$ FRET coupling range to decreasing emission. Thus long repeat sequences are not detected and other repeats may be ambiguously recognized. Photobleaching resolves this problem. Another problem with FRET is avoiding photobleaching of the donor molecules; this can be accomplished if it is the template DNA that is attached and the donor bearing DNA polymerase is periodically replaced.**

Korlach et al 1999 Provisional at Page 15, paragraphs 1.

Several points are worth noting: 1) the Korlach et al. 1999 Provisional **does not disclose the use of beta or gamma labeled dNTPs**; 2) like Schneider et al., the Korlach et al. 1999 Provisional FRET strategy is concerned about fluorophores on the formed DNA duplex; 3) the 60Å or 50 Å teaching is not directed to donor tag placement to control inter-tag separation during monomer incorporation, but this teaching is related to how quickly an incorporated fluorophore would no longer contribute to a FRET signal; 4) like Schneider et al., the Korlach et al. 1999 Provisional FRET strategy discloses that photobleaching would be one way to improve the problem of multi-label FRET signals; and 5) the Korlach et al. 1999 Provisional discloses nothing on how to tag the polymerase, save the a donor is attached to the polymerase.

Moreover, **Schneider et al. more clearly disclosed the use of labeled polymerases and labeled nucleotides in a FRET sequencing strategy,** ***but made absolutely no mention of beta***

or gamma labeled dNTPs whatsoever. Rather their invention focused solely on nucleotides labeled on the base, sugar or backbone phosphate, *i.e.*, persistently labeled nucleotides.

Furthermore, Applicants still believe that most researchers at the time of filing of Applicants' application did not believed that nucleotides modified at the gamma phosphate would produce extension products – rather most researchers believed they would act as terminators of enzyme activity. The following text from Kao et al. supports this belief:

> Several companies sell products that incorporate a detectable reagent into the product of polymerase synthesis, including Boehringer (Genius kit), Life Technologies INC., GIBC/BRL, Sigma (biotinylated nucleotides, fluorescent nucleotides), Molecular Probes Inc. (a large range of fluorescent and caged nucleotides), Li-Cor (dyes attached to DNAs for DNA sequencing), etc. Reports of γ-phosphoesters of nucleoside triphosphates have described them as non-hydrolyzable and used them in solid phase affinity purification protocols, e.g. Clare M. M. Haystead, et al., Gamma-phosphate-linked ATP-Sepharosefor the affinity purification of protein kinases, Eur. J. Biochem. 214, 459-467 (1993), esp. p.460, col. 2, line 23. We synthesized large numbers of γ-phosphoester nucleoside triphosphates and found that while they are indeed non-hydrolyzable by many enzymes, they are often suitable substrates for RNA and DNA polymerases.

US 6,399,335 at Col. 1, ll. 31-47.

Based on the disclosure in Kao et al., Applicants believe that ordinary artisans would not consider such labeled nucleotides as good monomers for making a DNA strand, and clearly not an obvious choice for labeling if you want the polymerase to continue DNA synthesis.

It is clear from the teaching of the Korlach et al. 1999 Provisional and Schneider et al., that two groups of experts in this area did not discover a tag interactive strategy involving detecting interactions between tagged dNTPs, where the tag is on the pyrophosphate portion of the nucleotide and is released upon incorporation, and a tagged polymerase, regardless of whether the interaction is FRET or direct detection of the fluorescent properties of one or both of the tags. If two groups of leading experts in the area did not recognize this invention, no one can expect an ordinary artisan to have recognized this invention either. Thus, the fact that these two references fail to disclose sequencing strategies involving non-persistently tagged dNTPs (tagged on the pyrophosphate moiety) and tagged polymerases is strong evidence in support of patentability of the claims of this invention.

Conclusions

1. Applicants' proposal is sufficient to antedate the Korlach et al. 2000 Non-Provisional.
2. The Korlach et al. 1999 Provisional does not disclose beta or gamma labeled dNTPs.
3. The present claims are patentable over the Korlach et al. 1999 Provisional with or without Schneider et al., because the Korlach et al. 1999 Provisional alone or in combination with Schneider et al. do not discloses sequencing strategies involving non-persistenly tagged dNTPs and tagged polymerases.

The Commissioner is authorized to charge or credit deposit account 501518 for any fees due or any overpayments, respectively.

If the Examiner requires additional information, then Applicants request that the Examiner contact their Attorney, Robert W. Strozier, at 713-977-7000.

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Respectfully submitted,

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